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PRINCIPAL INVESTIGATOR: Margarete K. Akens, Ph.D.

CONTRACTING ORGANIZATION: Sunnybrook Health Sciences Centre
Toronto, ON, Canada M4N 3M5

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6. AUTHOR(S)

Dr. Margarete K. Akens
Dr. Cari M. Whyne
Dr. Brian C. Wilson, Dr. Albert J.M. Yee, Dr. Diane Nam
E-Mail: makens@sri.utoronto.ca

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Toronto, ON, Canada M4N 3M5

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14. ABSTRACT

Long bone fractures resulting from high impact trauma can result in delayed healing. Photodynamic therapy (PDT) is a non-surgical, non-ionizing minimally invasive local treatment currently used to treat cancer and skin diseases. Surprisingly, recent findings from studying the effect of PDT on spinal metastases have shown that PDT improved the strength and stiffness of the vertebrae. Although variability in fracture generation led to inconclusive findings with respect to bone generation in the closed tibia fracture model (n=17), higher local levels of VEGF were found in PDT treated animals. A critical size defect fracture of the femur was generated in 20 adult female Sprague-Dawley rats. PDT treatment was applied either 1d (n=6) or 7d (n=6) after fracture generation. Qualitatively, a larger callus was seen in the 7d-PDT group, suggesting an important effect of timing on PDT administration. Both PDT treated groups showed evidence of healing and closing of the fracture gap in this critical defect model. Preliminary, quantitative μCT measurements support the qualitative findings. Additional μCT based imaging and stereological analysis are underway. Histologic processing and analysis of the serum will also be conducted on these animals.

15. SUBJECT TERMS
Long bone fractures, Photodynamic therapy, Fracture healing, Animal model;

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Photodynamic Therapy Treatment to Enhance Fracture Healing

Introduction……..……..…..

Long bone fractures resulting from high impact trauma pose a continuous challenge to successful fracture healing. Even though a variety of treatments are currently available to reduce the risk of infection and/or enhance bone healing, it still can take up to a year for these fractures to heal. Photodynamic therapy (PDT) is a non-surgical, non-ionizing minimally invasive treatment, which combines the administration of a photosensitizing drug with the application of light and is currently used to treat cancer and skin diseases. Unexpectedly, recent findings from studying the effect of PDT on the vertebrae when treating spinal metastases have shown that PDT rapidly improved vertebral bone strength, stiffness and architecture [1]. Based on these observations, the aim of this study is to explore the ability of PDT as a novel treatment approach to enhance fracture healing.

Body……..

Task 1: Closed tibial fractures were created in a total of 17 rats using the drop weight system following intramedullary pinning. While modifications to the drop weight apparatus improved the consistency of the generated fractures, additional analyses have demonstrated that the inherent variability in the closed model may mask the treatment effects of PDT being studied. To date μCT analyses and histologic assessment have been conducted on 17 tibiae. The increase in bone formation after PDT treatment observed in the first group of 11 rats in the μCT analyses were not present in the second group of 6 rats (n=2 control; n=2 1d-PDT; n=2 7d-PDT). However, the slight increase in local VEGF protein expression after PDT treatment as determined through positive pixel count (Positivity = Npositive/NTotal) remained (control (n=6) 51±5 %; 1dPDT (n=5) 55±5 %; and 7dPDT (n=6) 56±7 %). In contrast, VEGF levels in the serum were found to be lower in the PDT treated groups (control (n=2) 57±5.5 pg/ml; 1dPDT (n=2) 35.2±14.8 pg/ml and 7dPDT (n=2) 33.0±13.7 pg/ml). A systemic elevated and prolonged VEGF level has been found as an indication of impaired bone healing in patients with long bone fractures[2]. Based on these relatively inconclusive results found in the closed fracture model the focus was shifted to the more repeatable open critically sized defect fracture model.

Task 2: The development of a robust critical size defect fracture model in rats has been accomplished using the RatFix plating system (RISystem, Davos, Switzerland). The RatFix system consists of an 8-hole PEEK plate secured to the proximal and distal femoral shaft with a total of 6 titanium screws. Following the plate fixation, a 6 mm piece of bone is removed mid-

shaft using a Gigli saw (Figure 1). The 6 mm gap distance is considered to be a critical size defect in rats which will not heal [3]. The variability of this model is much lower compared to the closed fracture model described above. All rats have received antibiotics and analgesics and tolerated the procedure and PDT treatments well.

Similar to the closed fracture model, animals were randomly allocated to 3 groups: control (no treatment); PDT applied 1 day (1d) post fracture or PDT applied 7 days (7d) post fracture (Figure 2). High resolution x-ray images taken on day 1 confirmed the fracture generation and the positioning of the plates (Figure 3A). Follow up in vivo imaging is continued to 6 weeks post-surgery (Figure 3B). To date a total of 20 animals have been treated. Of these, 2 animals in the first group (1 control animal and 1 from the 7d-PDT group) and 1 animal in the second group (directly following surgery due to a surgical error) had to be euthanized early due to loosening of the plate due to a technical problem. These problems have been resolved and all other 17 animals have experienced no hardware related or technical issues. The earlier problem with serum preservation has also been solved and the serum from all animals is being collected and stored for analysis at -20 °C.

Six rats have been euthanized 7 weeks after fracture generation and the femora harvested for further analyses. Qualitative analysis of both total volume of the fracture site (TV - including the callus formation) and bone volume within the callus (BV) appeared higher in the 7d-PDT group, suggesting a treatment effect and the importance of timing on PDT administration. Micro CT images at an isotropic 13 μm^3 voxel resolution (Inveon CT, Siemens, Erlangen, Germany) were acquired of the fracture site and callus for 3D architectural analysis (Amira, Berlin, Germany). Scanning has been optimized to reduce artifacts in the fracture gap caused by the titanium screws. Initial structural analysis has focused on TV, BV and the gap distance. µCT imaging and quantitative analysis of the fracture site has been completed on 2 femora. One femur in the 1d-PDT group had a TV = 12 mm^3, BV = 8.9mm^3 and a gap distance of 3.6 mm and the other femur in the 7d-PDT group had a TV = 23 mm^3, a BV = 18.9mm^3 and a gap distance of 3.5mm. These femora are currently being processed for histology. The other four rat femora are awaiting µCT-scanning and 11 more rats are in progress. One group (n=5) will be sacrificed this week and a second group (n=6) will be sacrificed in mid-January 2013.

Key Research Accomplishments……..……..

- A local increase in VEGF expression was found in the 7d-PDT group in the closed tibia fracture model with a concurrent decrease in systemic VEGF serum levels.
- Establishment of a repeatable rat femur critical size defect fracture model

- Successful application of PDT to the critically sized defects with preliminary results indicating a positive timing dependent effect on fracture healing.

Reportable Outcomes……

- Podium presentation at the Canadian Orthopaedic Research Society (CORS) Annual Meeting; 08. June 2012 in Ottawa, ON, Canada
- Poster presentation at the Military Health System Research Symposium (MHSRS); 13.-16. August 2012 in Fort Lauderdale, FL

The Effect of Photodynamic Therapy (PDT) on Long Bone Fracture Healing

+[1,3]Akens, M.K.; [1,3]Wright, D.; [2]Yousef, S.; [1,3]Nam, D., [1,3]Yee, A.J.M, [2,3]Wilson, B.C., [1,3]Whyne, C.M.
+[1]Orthopaedic Biomechanics Lab, Sunnybrook Research Institute, [2] Ontario Cancer Institute, University Health Network, [3]University of Toronto, Toronto, ON, Canada

Conclusion……

Despite the variability of the closed tibia fracture model leading to inconclusive results with respect to bone healing following PDT, the increase in local VEGF and concurrent decrease of VEGF in the serum indicates a potential positive role of PDT in fracture healing. In the critical sized defect model more bone formation is observed qualitatively when PDT is applied at the secondary stage of fracture healing compared to PDT applied at day 1. If ongoing quantitative analyses confirm the benefits of PDT treatment at the secondary stage of fracture healing, PDT could be applied to trauma patients expected to encounter impaired healing, even without access to immediate medical care, as is often the case in military situations.

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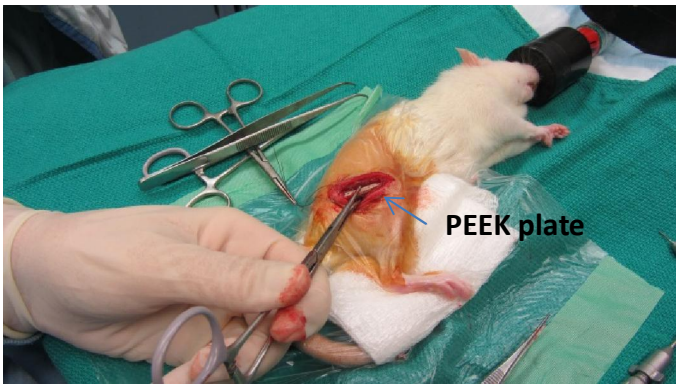
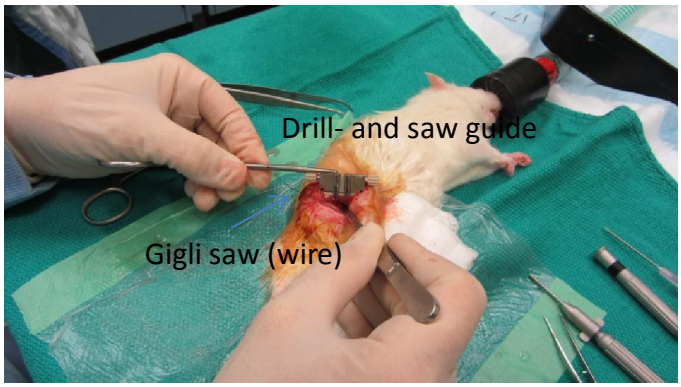
Appendices……..

Figures

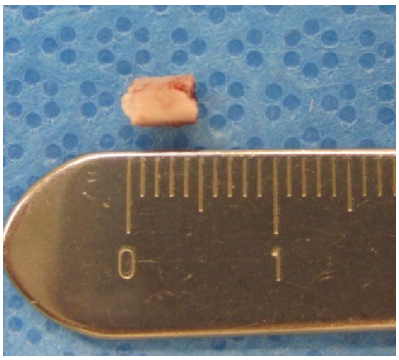
Figure 1: Critical size defect fracture model in rat femur



Positioning of the PEEK plate on to the right femur of a Sprague Dawley rat.



Positioning of the drill- and saw guide on top of the secured plate prior to removing bone using a wire.



Bone removed from the diaphysis of the femur.

Figure 2: PDT treatment

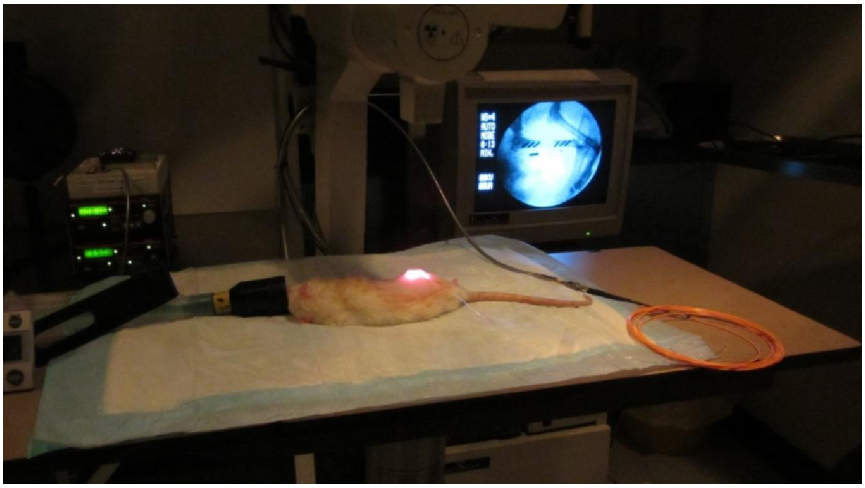


Figure 3: High resolution x-ray imaging of the healing femora